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ORIGINAL INVESTIGATION

Tatiana Lipina · Viviane Labrie · Ina Weiner · John Roder

Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia

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Abstract *Rationale:* Schizophrenia is characterized by disturbances in sensorimotor gating and attentional processes, which can be measured by prepulse inhibition (PPI) and latent inhibition (LI), respectively. Research has implicated dysfunction of neurotransmission at the NMDA-type glutamate receptor in this disorder. *Objectives:* This study was conducted to examine whether compounds that enhance NMDA receptor (NMDAR) activity via glycine B site, D-serine and ALX 5407 (glycine transporter type 1 inhibitor), alter PPI and LI in the presence or absence of an NMDAR antagonist, MK-801. *Methods:* C57BL/6J mice were tested in a standard PPI paradigm with three prepulse intensities. LI was measured in a conditioned emotional response procedure by comparing suppression of drinking in response to a noise in mice that previously received 0 (non-preexposed) or 40 noise exposures (preexposed) followed by two or four noise–foot shock pairings. *Results:* Clozapine (3 mg/kg) and D-serine (600 mg/kg), but not ALX 5407, facilitated PPI. MK-801 dose dependently reduced PPI. The PPI disruptive effect of MK-801 (1 mg/kg) could be reversed by clozapine and ALX 5407, but not by D-serine. All the compounds were able to potentiate LI under conditions that disrupted LI in controls. MK-801 induced abnormal persistence of LI at a dose of 0.15 mg/kg. Clozapine, D-serine, and ALX 5407 were equally able to reverse persistent LI induced by MK-801. *Conclusions:* D-Serine and ALX 5407 display similar effects to clozapine in PPI and LI mouse models, suggesting potential neuroleptic action. Moreover, the finding that agonists of NMDARs and clozapine can restore disrupted LI and disrupt persistent LI may point to a unique ability of the NMDA system to regulate negative and positive symptoms of schizophrenia.

Keywords Prepulse inhibition · Latent inhibition · C57BL/6J mice · D-Serine · ALX 5407 · Clozapine · MK-801 · Schizophrenia

T. Lipina (✉) · V. Labrie · J. Roder
Samuel Lunenfeld Research Institute,
Mount Sinai Hospital,
600 University Avenue,
Toronto, Ontario, M5G 1X5, Canada
e-mail: lipina@mshri.on.ca
Tel.: +1-416-5868242
Fax: +1-416-5868588

V. Labrie · J. Roder
Program in Neuroscience,
University of Toronto,
Toronto, Ontario, Canada

I. Weiner
Department of Psychology,
Tel Aviv University,
Tel Aviv, Israel

Introduction

Recent genetic linkage studies have implicated susceptibility genes for schizophrenia (see Scheffer 2002, for a review), some of which perturb the glutamate signaling pathways (Goff and Coyle 2001; Lewis et al. 2003; Harrison et al. 2003). A role for glutamate in schizophrenia is strongly indicated by clinical observations that dissociative anesthetics, such as phencyclidine (PCP) or ketamine, produce schizophrenia-like psychosis in healthy humans and exacerbate symptoms in schizophrenic individuals (Javitt and Zukin 1991; Krystal et al. 1994). Dissociative anesthetics bind to sites within the NMDA receptor (NMDAR) channel, acting as noncompetitive, use-dependent antagonists. It was shown that the serum concentration of PCP able to produce psychiatric symptoms corresponds to the level that blocks NMDARs (Javitt and Zukin 1991). Unlike the indirect dopamine agonist amphetamine, that produces only positive symptoms of schizophrenia, NMDAR blockers generate negative symptoms and cognitive impairments characteristic of the disorder. Furthermore, in PET scans of normal humans, a subanesthetic dose of ketamine increased amphetamine-induced dopamine release to an extent that mimicked the exaggerated response seen in schizophrenic subjects (Kegeles et al. 2000). These results suggest that psychosis (i.e., positive symptoms) and changes in dopamine release may be secondary to a primary defect in the regulatory

corticolimbic glutamatergic neuronal pathway (Coyle et al. 2003). Hence, it has been theorized that schizophrenia may be associated with NMDAR hypofunction and, by corollary, that enhancement of NMDAR function may be beneficial in treating this disorder (Krystal et al. 1994; Goff and Coyle 2001; Coyle et al. 2003).

A unique characteristic of the NMDAR is that it contains, in addition to a binding site for the agonist, glutamate, a coagonist site called the glycine modulatory site (GMS) to which glycine and D-serine can bind. Both binding sites must be occupied in order to activate the channel. Exogenous administration of glycine and D-serine can stimulate the NMDAR without leading to the toxic effects observed following the administration of direct NMDAR agonists such as glutamate and NMDA (Coyle et al. 2003). D-Serine is a more potent activator than glycine of the GMS (Berger et al. 1998). Because exogenously administered glycine and D-serine must overcome potent regulatory brain mechanisms in order to effectively potentiate NMDAR-mediated neurotransmission, recent focus has been on increasing brain glycine and D-serine availability through regulation of clearance mechanisms. Mechanisms underlying glycine regulation are better understood. Molecular, biochemical, and behavioral findings indicate that local concentrations of glycine in the forebrain are tightly regulated by the actions of the high-affinity glycine transporter type 1 (GlyT1) (Smith et al. 1992; Berger et al. 1998; Atkinson et al. 2001). Therefore, selective inhibition of GlyT1 may produce a more potent enhancement of NMDAR activity than that obtained with exogenous glycine/D-serine administration.

Compounds that enhance activity at the GMS site, including glycine, D-serine, the partial co-agonist d-cycloserine, and glycine transport inhibitors, have been shown to reverse NMDA-antagonist-induced behavioral effects in humans and rodents (e.g., Toth and Lajtha 1986; Tanii et al. 1994; Javitt et al. 1999; Krystal et al. 2003). In addition, glycine, D-serine, d-cycloserine, and the GlyT1 inhibitor sarcosine reduced negative symptoms and improved cognitive function in patients with schizophrenia (Waziri 1988; Goff et al. 1999; Heresco-Levy 2003; Tsai et al. 2004; Heresco-Levy and Javitt 2004). Both D-serine and sarcosine also reduced positive symptoms, which may reflect their greater intrinsic potency (Coyle et al. 2003). A core characteristic of NMDAR-antagonist-induced behavioral deficits is their selective reversal by atypical but not typical antipsychotics (Malhotra et al. 1997; Swerdlow et al. 1998; Nilsson et al. 2001). Notably, the prototypical atypical neuroleptic, clozapine, has been reported to act as a partial agonist at GMS (Arvanov et al. 1997; Schwieler et al. 2004), suggesting that its therapeutic efficacy could in part be due to increased activation of the NMDA–glycine site.

Animal models of schizophrenia are an important means for testing novel pharmacological strategies for the treatment of this disorder. Prepulse inhibition (PPI) and latent inhibition (LI) are two animal models currently dominating neuropharmacological research of schizophrenia. Both have a reasonable amount of face, predictive, and construct validity.

PPI is a measure of sensorimotor gating and refers to the attenuation of the startle response by a weak stimulus (prepulse) appearing a short time prior to the startle stimulus. Deficits in PPI have been reported in schizophrenia (Swerdlow et al. 1994; Braff et al. 2001), and the degree to which PPI is affected correlates with symptom severity (Swerdlow et al. 1994). In rodents, PPI is disrupted by direct and indirect dopamine (DA) agonists as well as by NMDAR antagonists (for a review, see Geyer et al. 2001). The DA agonist and the NMDAR-antagonist-induced PPI deficits show a differential responsiveness to typical and atypical neuroleptics. The former can be reversed by both typical and atypical neuroleptics (Swerdlow et al. 1994), whereas atypical antipsychotics are more potent than typical ones in reversing the latter (Yamada et al. 1999). Although most studies have focused on the reversal of pharmacologically induced PPI disruptions, there are reports of PPI potentiating effects of antipsychotics in rats (Geyer et al. 2001) and mice (Olivier et al. 2001; Ouagazzal et al. 2001). To date, limited work has been done with agents enhancing NMDAR function. It has been reported that they enhance PPI when given on their own (Kinney et al. 2003) and reverse disrupted PPI caused by neonatal ventral hippocampal lesion (Le Pen et al. 2003).

LI refers to the proactive interference of nonreinforced stimulus preexposure with the capacity of that stimulus to acquire behavioral control when it is subsequently paired with reinforcement and indexes the capacity to downgrade the behavioral control of stimuli that predict no significant consequences. LI is disrupted in rats and normal humans treated with amphetamine and in the acute stages of schizophrenia (Weiner et al. 1984, 1988, 1996; Gray et al. 1992; Thornton et al. 1996; Rascle et al. 2001). Both typical and atypical neuroleptics reverse amphetamine-induced disruption of LI and reliably potentiate LI under conditions that normally do not yield robust LI (for reviews, see Weiner 1990, 2003; Weiner and Feldon 1997; Moser et al. 2000). Unlike amphetamine, acute administration of NMDAR antagonists spares LI (Weiner and Feldon 1992; Robinson et al. 1993; Turgeon et al. 2000). Recently, Gaisler-Salomon and Weiner (2003) showed that NMDAR antagonism induces an abnormally persistent LI that becomes manifested under conditions not yielding LI. As found with other NMDAR-antagonist-based models, persistent LI was reversed by clozapine and risperidone, but not by haloperidol (Gaisler-Salomon and Weiner 2003). In addition, it was normalized by glycine and d-cycloserine (Gaisler-Salomon and Weiner, unpublished observations).

The present experiments tested the effects of NMDAR glycine site modulators, D-serine and the high-affinity GlyT1 inhibitor ALX5407, on PPI and LI in C57BL/6J mice and compared them to the effects of the prototype atypical neuroleptic, clozapine. In both models, we tested (1) the effects of these agents given on their own on low levels of PPI and LI in order to determine whether they would potentiate PPI and LI. Basal PPI levels are relatively low in the C57BL/6J strain (Ouagazzal et al. 2001); reduced LI (in controls) was attained by appropriate parametric manipulation; (2) the effects of MK-801 on PPI and

LI; and (3) the capacity of D-serine, ALX5407, and clozapine to reverse MK-801-induced PPI and LI abnormalities (disrupted PPI and persistent LI, respectively).

Materials and methods

Subjects

C57BL/6J male mice were purchased from Jackson Laboratory (Bar Harbor, ME) and were 6–8 weeks old upon arrival. Animals were kept for at least a week in the animal colony at the Samuel Lunenfeld Research Institute prior to the beginning of behavioral testing. They were housed four per cage under a 12-h light/dark cycle (lights on at 0700 hours) with ad libitum food (Purina mouse chow) and water, except for the duration of the LI experiments (see below). All behavioral testing was conducted between 0900 and 1600 hours. The research and animal care were performed according to CACC guidelines. In both PPI and LI, each experimental group had n=7–10. Mice were randomized with regard to day and treatment and were only used once.

Apparatus and procedure

Prepulse inhibition

PPI testing was conducted in four foam-lined (sound-damping) isolation chambers (Startle Reflex System, MED Associates, St. Albans, VT; ENV-022s). Each chamber was equipped with an acoustic stimulator (ANL-925), a platform with a transducer amplifier (PHM-255A and PHM-250B), and an animal holder that was situated on the top of the platform and was large enough to allow the animal adequate movement. The holder had holes along the sides for ventilation. A fan and a red light were provided inside the chamber for the comfort of the animal when inside the enclosed chamber. PPI holders were cleaned with 70% ethanol between mice when PPI was finished. All events were recorded and controlled by MED Associates software (Startle Reflex package).

During the test the animal was confined to the holder. Background noise was set at 65 dB. Five types of trials were used. Pulse-alone trials (P) consisted of a single white noise burst (120 dB, 40 ms). The prepulse + pulse trials (PP69P, PP73P, and PP81P) consisting of a prepulse of noise (20 ms at 69, 73, or 81 dB, respectively) followed 100 ms after prepulse onset by a startling pulse (120 dB, 40 ms). No-stimulus (NS) trials consisted of background noise only. Sessions were structured as follows: (1) 15-min acclimation at background noise level; (2) five P trials; (3) ten blocks each containing all five trials (P, PP69P, PP73P, PP81P, and NS) in pseudorandom order; and (4) five P trials. Intertrial intervals were distributed between 12 and 30 s. The force intensity for each trial was recorded as the startle level. The average percent reduction in startle intensity between pulse and prepulse + pulse trials at all three prepulse levels was defined as the PPI level.

The percentage PPI induced by each prepulse intensity was calculated as [1 −(startle amplitude on prepulse trial)/(startle amplitude on pulse alone)]×100%. Startle magnitude in this formula was calculated as the average response to all of the P trials, excluding the first and last blocks of five P trials.

Latent inhibition

The LI procedure was modeled after Weiner (2001) (see also Gould and Wehner 1999). Several pilot studies were performed in order to determine the optimal parameters of preexposure and conditioning. In particular, we needed to establish parameters that yielded LI in normal mice as well as parameters that did not yield LI, in order to be able to demonstrate persistent or potentiated LI.

LI was measured in three conditioning chambers (MED Associates), each enclosed in a melamine sound-attenuating chamber (ENV-022M). The interior of the chamber was white with a speaker and a switch-control light bulb (ENV-221CL) mounted on the ceiling. Ventilation fans on the backs of the chests provided air exchange and background noise (68 dB). The conditioning chambers had clear Plexiglas walls (ENV-307W) and removable floors consisting of either metal rods, used on preexposure and conditioning days, or a flat piece of aluminum, used on pretraining, baseline drinking, and test days, and were equipped with a bottle with a metal tip (sipper tube). On the preexposure and conditioning days, access to the bottle was prevented by a guillotine door.

Foot shock of 1-s duration and 0.37-mA intensity was administered via the metal rods of the grid floor wired to a shock generator (ENV-414) via a scrambler. The auditory stimulus was an 85-dB white noise (ENV-324M). Licks were detected by a lickometer (ENV-350CM). When the mouse made contact with the floor of the chamber and the sipper tube (metal tip on regular bottle), a computer counted that as a lick. An IBM-PC compatible computer running MED Associates software (MED-PC) and connected to the chambers via an interface package (DIG-716P1 and ANL-926) controlled the administration of training and testing stimuli. All events were programmed by MED-PC software. The chambers were cleaned with 70% ethanol between sessions.

Prior to the beginning of each LI experiment, mice were weighed and water was removed from the cages for 24 h. They were then trained to drink in the experimental chamber for 5 days, 15 min/day (training period). Body weights were monitored daily throughout all behavioral testing and maintained at no lower than 80% of the initial body weight. On each daily training session, mice were acclimated to the chamber without access to the sipper tube for 5 min, then the guillotine door was opened. Latency to the first lick and number of licks were recorded for 15 min. The LI procedure was conducted on days 6–9 and consisted of the following stages.

Preexposure

The preexposed (PE) mice received 40 white noise presentations with an interstimulus interval of 60 s. The nonpreexposed (NPE) mice were confined to the chamber for an identical period of time without receiving the stimuli.

Conditioning

All mice received fear conditioning to the noise stimulus. In experiment 4, two levels of conditioning were used: one producing LI, namely, two noise–shock pairings, and one disrupting LI, namely four noise–shock pairings. In all the subsequent experiments, four pairings were used. The noise–shock pairings were given 5 min apart. Shock immediately followed noise termination. The first noise–shock pairing was given 5 min after the start of the session. After the last pairing, mice were left in the experimental chamber for an additional 5 min. After preexposure and conditioning, mice received 15-min access to water in their home cages.

Lick retraining

Mice were given a 15-min drinking session as during the training period. Data of mice that failed to complete 100 licks were dropped from the analysis.

Test

Each mouse was placed in the chamber with access to the sipper tube. When the mouse completed 75 licks the noise was presented and lasted until the mouse reached lick 101. The following times were recorded: time to first lick, time to complete licks 50–75 (before noise onset; A period) and time to complete licks 76–101 (after noise onset; B period). Degree of lick suppression was calculated as a suppression ratio $A/(A+B)$. A lower suppression score indicates a stronger suppression of drinking. LI consists of lower suppression of drinking (higher suppression ratio) in the preexposed compared to the nonpreexposed mice.

Drugs

Clozapine (Tocris, USA) was dissolved in NaCl 0.9% containing 0.3% Tween. D-Serine (Sigma, Canada), L-serine (Sigma) and MK-801 maleate salt (Sigma) were dissolved in saline (0.9% NaCl). ALX 5407 ((R)-*N*-[3-(4′-fluorophenyl)-3(4′-phenylphenoxy)propyl]sarcosine hydrochloride; Sigma) was dissolved in a solvent containing 25% 2-hydroxypropyl-β-cyclodextrin and 75% water, pH adjusted to ~6 using 1 N NaOH. Clozapine, MK-801 maleate, and ALX 5407 were injected intraperitoneally. D-Serine and L-serine were injected subcutaneously. Injection-testing interval was 30 min for clozapine, 15 min for MK-801, 20 min for D- and L-serine, and 120 min for ALX 5407. Doses of clozapine, D-serine, L-serine, and ALX 5407 are expressed as free base; doses of MK-801 are expressed as salt. All drugs were administered in a volume of 10 ml/kg. In LI experiments, all drugs were administered in the preexposure and conditioning stages.

Experimental design and statistical analysis

PPI (experiments 1–3)

Experiment 1 tested the effects of clozapine (3 and 6 mg/kg), D-serine (300, 600, and 900 mg/kg), L-serine (600 mg/kg), and ALX 5407 (1, 10, or 15 mg/kg) on PPI and startle amplitude. The doses of clozapine and ALX 5407 were chosen based on the PPI literature (Olivier et al. 2001; Ouagazzal et al. 2001; Kinney et al. 2003). D-Serine and L-serine doses were chosen based on other behavioral data (Nilsson et al. 1997) and results of pilot studies.

Experiment 2 tested the effects of MK-801 (0.15, 0.3, 0.6, and 1 mg/kg) on PPI. MK-801 doses were chosen based on the PPI literature (Curzon and Decker 1998; Yee et al. 2004).

Experiment 3 tested the effects of (1) clozapine (3 mg/kg), (2) D-serine (600 mg/kg), and (3) ALX 5407 (1 mg/kg) at doses chosen on the basis of the results in experiment 1 on disrupted PPI induced by 1 mg/kg MK-801. Each pretreatment–treatment combination included four groups in a 2×2×(3) factorial design with main factors of pretreatment (vehicle, clozapine; vehicle, D-serine; and vehicle, ALX 5407) and treatment (vehicle, MK-801) and a repeated measurement factor of prepulse (69, 73, and 81 dB).

Analysis of PPI data

The percentage of inhibition of startle and basic startle response for the different types of trials was analyzed with two-way ANOVAs with drug dose as a between-subjects factor, or with treatment and pretreatment as between-subjects factors and prepulse intensity as a repeated measurement factor. Fisher least significance difference test (LSD) was used for post hoc comparisons when ANOVAs yielded statistically significant main effects or interactions.

LI (experiments 4–7)

Experiment 4 tested LI with two levels of conditioning, two and four noise–shock pairings, in normal mice. It included four experimental groups in a 2×2 design with the main factors of PE (0, 40) and number of pairings (2, 4).

Experiment 5 tested the effects of clozapine (3 mg/kg), D-serine (600 mg/kg), and ALX 5407 (1 mg/kg) on LI with parameters of extended conditioning. The doses used were those found effective in PPI experiments. The experiment included eight experimental groups in a 2×4 design with the

main factors of PE (0, 40) and drug treatment (vehicle, clozapine, D-serine, and ALX 5407).

Experiment 6 tested the effects of MK-801 (0.05, 0.1, 0.15, and 0.2 mg/kg) on LI with parameters of extended conditioning. The doses of MK-801 were based on Gaisler-Salomon and Weiner's (2003) results in rats. The experiment included ten experimental groups in a 2×5 design with the main factors of preexposure (0, 40) and drug (vehicle, four doses of MK-801).

Experiment 7 tested the effects of clozapine (3 mg/kg), D-serine (600 mg/kg), and ALX 5407 (1 mg/kg) on persistent LI induced by 0.15 mg/kg MK-801. The experiment included ten experimental groups in a 2×5 design with the main factors of PE (0, 40) and drug (vehicle, MK-801, clozapine + MK-801, D-serine + MK-801, and ALX 5407 + MK-801).

Analysis of LI data

Times to complete licks 50–75 (A period) and suppression ratios were analyzed by two-way ANOVAs with main factors of preexposure (0, 40) and drug conditions (four levels in experiment 5, and five levels in experiments 6 and 7). Significant main effects and interactions were followed by LSD post hoc comparisons to assess the differences between PE and NPE groups within each condition.

Results

Experiment 1: effects of clozapine, D-serine, and ALX 5407 on startle amplitude and PPI

Table 1 shows PPI at the three prepulse intensities and startle amplitude following the administration of clozapine (3 and 6 mg/kg), D-serine (300, 600, and 900 mg/kg), L-serine (600 mg/kg), and ALX 5407 (1, 10, and 15 mg/kg).

Clozapine had a significant effect on startle ($F_{2,21}$=35.8, p<0.0001). Post hoc comparisons revealed a significant effect on startle at 6 mg/kg (p<0.0001), but not at 3 mg/kg dose. PPI analysis yielded significant main effects of prepulse intensity (69, 73, and 81 dB) ($F_{2,42}$=11.9, p<0.001) and drug ($F_{2,21}$=6.0, p<0.01) as well as their significant interaction ($F_{4,42}$=4.5, p<0.01). ANOVA revealed a significant main effect of clozapine treatment at 69 dB ($F_{2,21}$=6.2, p<0.01) and at 81 dB prepulse ($F_{2,21}$=4.1, p<0.05), but not at 73 dB. Post hoc analysis revealed a facilitatory effect of 3 mg/kg clozapine at 69 dB prepulse (p≤0.01) and at 81 dB (p<0.05; see Table 1).

D-Serine had no effect on startle amplitude ($F_{3,28}$=0.89, p>0.05). Analysis of PPI yielded a significant main effect of prepulse intensity ($F_{2,56}$=5.9, p<0.01) and a main effect of drug, which approached significance ($F_{3,28}$=2.5, p=0.055). ANOVA revealed a main effect of D-serine at 69 dB ($F_{3,28}$=2.6, p≤0.05), but not at 73 and 81 dB. Post hoc analysis revealed a significant effect of D-serine at a dose of 600 mg/kg at 69 dB prepulse (p<0.05; see Table 1). L-Serine at 600-mg/kg dose had no effect on startle ($F_{1,13}$=0.25, p>0.05). Analysis of PPI revealed only a main effect of prepulses ($F_{2,26}$=6.5, p<0.01).

ALX 5407 had no effect on startle ($F_{3,27}$=1.3, p>0.05), but there was a tendency for startle amplitude to increase at the highest dose (15 mg/kg) compared with controls (1,561.2±130.4 vs 1,245.7±78.2; p=0.08). Analysis of PPI revealed main effects of prepulse ($F_{2,54}$=4.9, p<0.01) and drug ($F_{3,27}$=2.9, p=0.055). Post hoc comparisons yielded a significant effect for 15 mg/kg ALX 5407 at 73- and 81-dB prepulses (p<0.05). At the dose of 10 mg/kg, ALX 5407 had a significant effect on all three prepulses (p<0.05), whereas no effect was observed at the 1-mg/kg dose (p>0.05; Table 1).

Table 1 Effects of clozapine, D-serine, L-serine, and ALX 5407 on prepulse inhibition (PPI) at three prepulse intensity levels and startle

	PPI 69 dB (%)	PPI 73 dB (%)	PPI 81 dB (%)	Startle response
Clozapine (mg/kg)				
Vehicle (n=8)	54.8±6.2	57.6±6.5	59.6±5.2	1,213.8±130.1
3.0 (n=8)	76.3±3.4**	70.7±3.5	75.5±3.8*	1,026.5±60.3
6.0 (n=8)	51.6±6.7	66.9±8.3	77.8±5.3	238.6±45.0***
D-Serine (mg/kg)				
Vehicle (n=8)	45.2±4.8	52.2±6.2	60.9±4.6	1,326.1±130.4
300 (n=7)	47.0±12.8	54.2±13.6	59.7±10.6	1,380.2±89.3
600 (n=8)	66.2±3.4*	64.0±4.4	70.5±4.0	1,123.3±131.7
900 (n=8)	43.8±9.2	52.4±8.1	51.3±9.2	1,414.4±202.3
L-Serine (mg/kg)				
Vehicle (n=8)	46.8±5.2	50.4±4.3	52.6±5.2	1,257.3±105.4
600 (n=7)	45.9±8.9	54.5±3.2	55.8±5.9	1,414.4±116.5
ALX 5407 (mg/kg)				
Vehicle (n=8)	59.4±3.3	64.4±3.8	68.4±2.9	1,245.7±78.2
1 (n=7)	51.1±7.7	56.2±5.5	64.6±6.2	1,292.0±165.8
10 (n=8)	41.0±7.8*	40.3±10.3*	44.5±11.6*	1,455.6±130.3
15 (n=8)	45.9±6.0	37.6±9.2*	44.2±7.9*	1,561.2±130.4

*p<0.05, **p<0.01, ***p<0.001 in comparison with vehicle-treated mice (post hoc LSD test, ANOVA)

Experiment 2: effect of MK-801 on startle amplitude and PPI

Table 2 summarizes the effects of MK-801 on startle amplitude. There was no effect of treatment on startle ($F_{4,35}$=1.2, p>0.05), although there was a trend toward increased startle amplitude at the highest dose (1 mg/kg: 1,568.5±85.9 vs 1,218.4±124.8).

Analysis of PPI revealed main effects of prepulse ($F_{2,70}$=45.7, p<0.001) and treatment ($F_{4,35}$=7.4, p<0.001) as well as a significant Prepulse×Treatment interaction ($F_{8,70}$=2.3, p<0.05). MK-801 at all doses inhibited PPI at all three prepulse intensities (see Fig. 1). ANOVA revealed a significant main effect of MK-801 at 69 dB prepulse ($F_{4,35}$=7.8, p<0.001), at 73 dB prepulse ($F_{4,35}$=5.9, p<0.001), and at 81 dB prepulse ($F_{4,35}$=4.7, p<0.01). Post hoc comparisons revealed a less pronounced effect of MK-801 at the 0.15-mg/kg dose (p<0.05 at all three prepulses) and at the 0.3-mg/kg dose (p<0.05 at 69 dB; p=0.08 at 73 dB, and p>0.05 at 81 dB, respectively). A more pronounced effect was revealed at the 0.6-mg/kg dose on the three prepulse intensities (69, 73, and 81 dB; p<0.001, p<0.01, and p<0.001, respectively). Maximal MK-801 effect was found at 1 mg/kg on all three prepulse intensities (p<0.001, p<0.001, and p<0.01, respectively).

Experiment 3: effects of clozapine, D-serine, and ALX 5407 on disrupted PPI induced by MK-801 and startle

Table 2 shows the mean startle amplitude in the five drug conditions: vehicle, MK-801, clozapine + MK-801, D-serine + MK-801, and ALX 5407 + MK-801. Three 2 (treatment)×2 (pretreatment) ANOVAs conducted on the mean startle amplitude in experiments 3A, B, and C, revealed only a significant main effect of MK-801 treatment on startle amplitude ($F_{1,28}$=5.34, p<0.05) in experiment 3A. None of the other main effects or interactions were significant (see Table 2).

Figure 2a–c depicts the effects of clozapine, D-serine, and ALX 5407, respectively, on disrupted PPI induced by 1 mg/kg MK-801. As can be seen in Fig. 2a, MK-801 disrupted PPI, whereas clozapine potentiated PPI. This was supported by significant main effects of treatment ($F_{1,28}$=32.9, p<0.001) and pretreatment ($F_{1,28}$=8.5, p<0.01). There were no significant Pretreatment×Treatment or Pretreatment×Treatment ×prepulse interactions, ($F_{1,28}$=1, 15, p>0.05) and ($F_{2,56}$=0.03, p>0.05), respectively.

As can be seen in Fig. 2b, MK-801 disrupted PPI (main effect of treatment: $F_{1,35}$=64, 3, p<0.001), and D-serine potentiated PPI (main effect of pretreatment: $F_{1,35}$=9.3, p<0.01). There were no significant interactions of Pretreatment×Treatment or Pretreatment×Treatment×Prepulse factors ($F_{1,35}$=0.9, p>0.05) and ($F_{2,70}$=2.7, p>0.05), respectively.

As can be seen in Fig. 2c, ALX 5407 reversed the MK-801-induced PPI deficit but had no effect on basic PPI. This was supported by the results of ANOVA, which yielded a main effect of treatment ($F_{1,28}$=12.7, p≤0.001) as well as significant Pretreatment×Treatment ($F_{1,28}$=9.2, p<0.01) and Pretreatment×Treatment×Prepulse ($F_{2,56}$=5.2, p<0.01) interactions, but no effect of pretreatment ($F_{1,28}$=2.7, p>0.05). Post hoc analysis revealed reversal of disrupted PPI after administration of ALX 5407 at 69 and 73 dB (p<0.01).

Table 2 Effects of MK-801, clozapine + MK-801, D-serine + MK-801, and ALX 5407 + MK-801 on startle

	Startle response
Vehicle (n=8)	1,218.4±124.8
0.15 mg/kg MK-801 (n=8)	1,344.1±174.6
0.3 mg/kg MK-801 (n=8)	1,389.6±168.4
0.6 mg/kg MK-801 (n=8)	1,150.2±177.0
1.0 mg/kg MK-801 (n=8)	1,568.5±85.9
Vehicle (n=8)	1,259.9±52.8
1.0 mg/kg MK-801 (n=8)	1,584.8±171.4*
Clozapine + MK-801 (n=8)	1,117.1±105.5
D-Serine + MK-801 (n=10)	1,288.2±114.0
ALX 5407 + MK-801 (n=8)	1,302.1±174.0

*p<0.05 in comparison with vehicle-treated mice (post hoc LSD test, ANOVA)

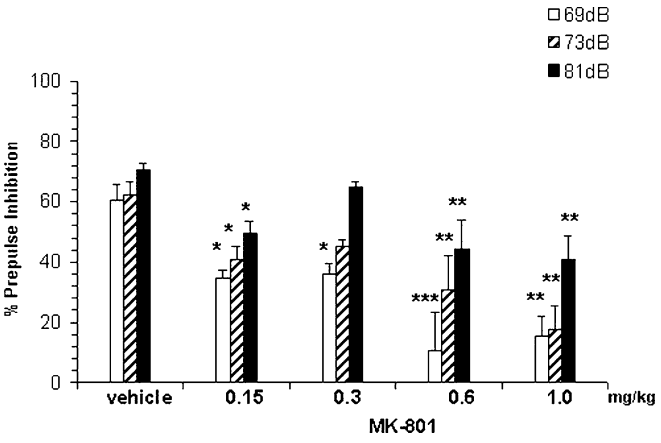

Fig. 1 The effect of MK-801 (n=8 per group) on prepulse inhibition at three prepulse intensity levels. *p<0.05, **p<0.01, ***p<0.001, in comparison with vehicle-treated group (post hoc LSD test, ANOVA)

Experiment 4: latent inhibition with two or four conditioning trials

Table 3 presents the mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in the four experimental groups. ANOVA of A periods revealed no differences between the four groups in their times to complete licks 50–75 before noise onset (all p's >0.05; overall mean A period=7.98 s). Figure 3 presents the mean suppression ratios of the PE and NPE groups conditioned with two or four pairings. As can be seen, LI was present with two but not with four trials. ANOVA revealed significant main effects of preexposure ($F_{1,29}$=11.6, p<0.01) and number of conditioning trials ($F_{1,29}$=7.9, p<0.01) as well as a significant Preexposure×Conditioning Trials in-

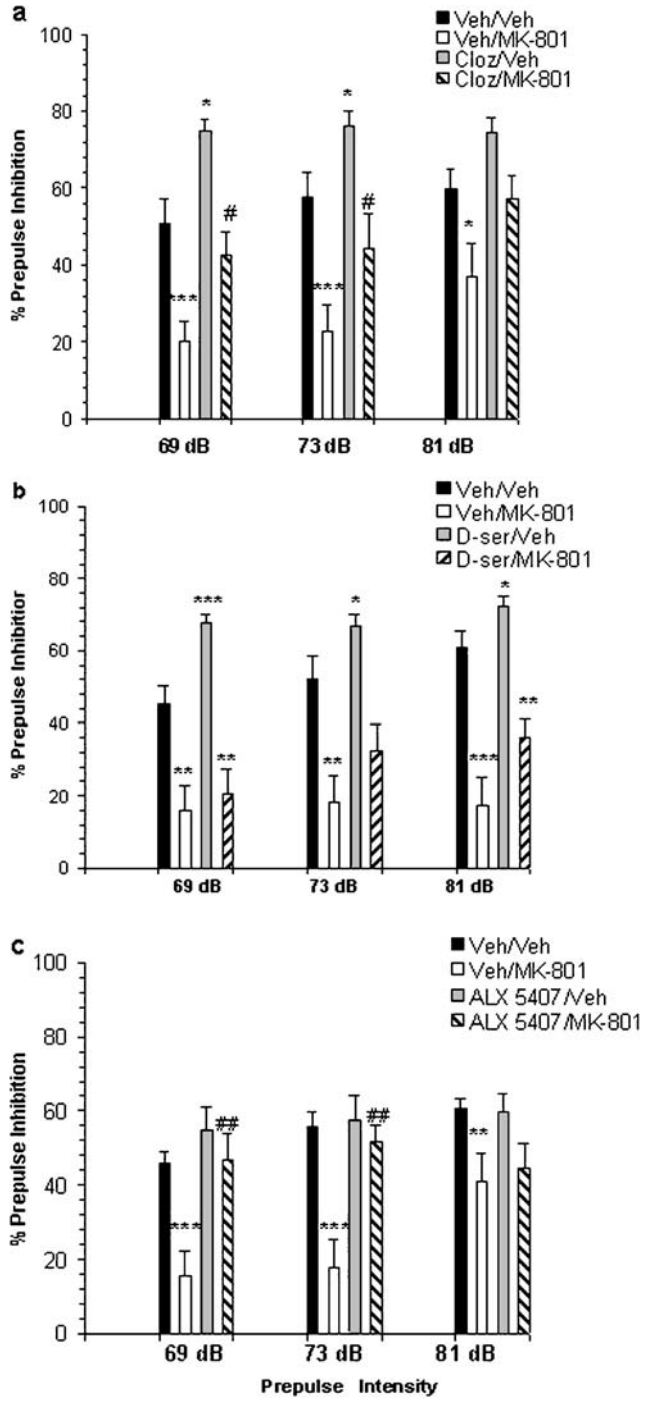

Fig. 2 The effects of clozapine (3 mg/kg; n=8 per group) (**a**), D-serine (600 mg/kg; n=9–10 per group) (**b**), and ALX 5407 (1 mg/kg; n=7–8 per group) (**c**) on MK-801 (1 mg/kg)-induced prepulse inhibition deficit. **p<0.01, ***p<0.001 in comparison with vehicle-treated group; #p<0.05, ##p<0.01, in comparison with MK-801-treated mice (post hoc LSD test, ANOVA)

teractions ($F_{1,29}$=13.2, p<0.001). Post hoc comparisons revealed a significant difference in suppression ratios between PE and NPE groups in the procedure with 40 PE and two conditioning trials (p≤0.01), but not in the procedure with 40 PE and four conditioning trials (p>0.05).

Table 3 Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in experiment 4

	A period	B period
2 CS–US		
PE	6.5±1.6	7.5±2.5
NPE	5.5±1.6	64.7±14.1
4 CS–US		
PE	10.8±5.3	65.5±28.1
NPE	9.1±1.8	101.1±23.2

Experiment 5: effects of clozapine, D-serine, and ALX 5407 on LI with 40 PE and four conditioning trials

Table 4 presents the mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in the eight experimental groups. ANOVA of A periods revealed no differences between the eight groups in their times to complete licks 50–75 before noise onset (all p's >0.05; overall mean A period=6.24 s). Figure 4 depicts the mean suppression ratios of the PE and NPE groups in the four drug conditions: vehicle, 3 mg/kg clozapine, 600 mg/kg D-serine, and 1 mg/kg ALX 5407. There was a significant main effect of preexposure ($F_{1,56}$=15.1, p<0.001), and drug ($F_{3,56}$=3.2, p<0.05), but no significant Preexposure× Drug interaction ($F_{3,56}$=2.2, p>0.05). Post hoc analysis revealed existence of LI in the 3 mg/kg clozapine (p<0.001), 600 mg/kg D-serine (p<0.01), and 1 mg/kg ALX 5407 conditions (p<0.05), but no LI in the vehicle condition (p>0.05).

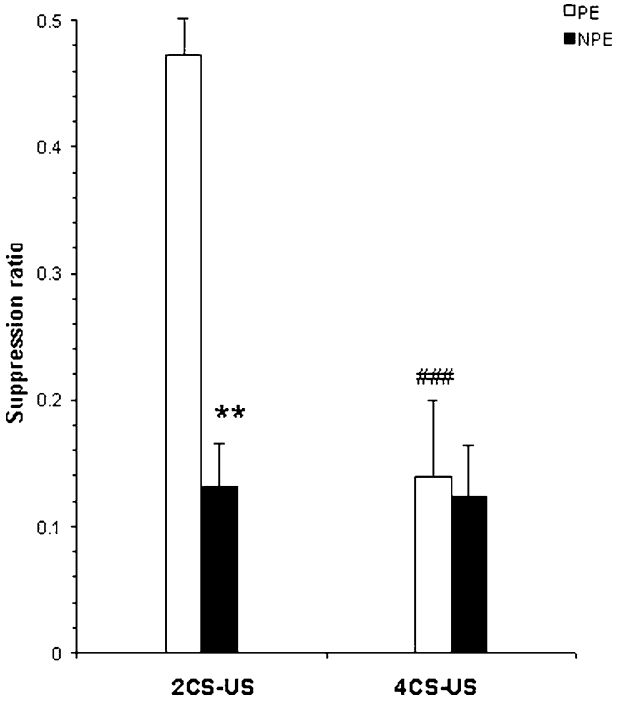

Fig. 3 Mean suppression ratios of the preexposed (*PE*) and nonpreexposed (*NPE*) mice conditioned with two (2CS–US; n=9 in PE and 8 in NPE groups, respectively) or four (4CS–US; n=8 per group) noise–shock pairings following 40 nonreinforced noise preexposures. **p<0.001, NPE in comparison with PE score; ###p<0.001, PE score with two CS–US in comparison with PE score with four CS–US (post hoc LSD test, ANOVA). CS-conditioned stimulus; US-unconditioned stimulus

Table 4 Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in experiment 5

	A period	B period
Vehicle		
PE	3.3±0.2	69.5±25.8
NPE	6.4±3.0	115.1±39.9
Clozapine		
PE	3.8±1.8	64.1±5.0
NPE	5.5±2.2	157.8±17.8
D-Serine		
PE	4.5±1.5	27.9±14.0
NPE	8.6±5.6	173.7±59.4
ALX 5407		
PE	12.6±2.4	37.7±17.7
NPE	5.2±2.2	41.8±11.8

Table 5 Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in experiment 6

	A period	B period
Vehicle		
PE	3.7±0.3	75.5±23.8
NPE	5.2±3.0	81.1±20.9
0.05 mg/kg MK-801		
PE	4.7±1.6	68.3±15.4
NPE	10.0±4.0	80.3±24.3
0.1 mg/kg MK-801		
PE	4.5±2.9	132.5±44.1
NPE	16.1±8.3	142.1±62.5
0.15 mg/kg MK-801		
PE	9.1±3.3	45.1±23.3
NPE	6.2±1.6	123.8±34.6
0.2 mg/kg MK-801		
PE	4.2±0.9	36.8±11.2
NPE	3.8±0.6	64.2±36.6

Experiment 6: effects of MK-801 on LI with 40 PE and four conditioning trials

Table 5 presents the mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in the ten experimental groups. ANOVA of A periods revealed no differences between the ten groups in their times to complete licks 50–75 before noise onset (all p's >0.05; overall mean A period=6.75 s). Figure 5 presents the mean suppression ratios of the PE and NPE groups in the five drug conditions: vehicle, 0.05, 0.1, 0.15, and 0.2 mg/kg MK-801. ANOVA yielded a significant Preexposure×Drug interaction ($F_{4, 68}$=3.5, p≤0.01). Post hoc analysis revealed a significant difference between PE and NPE groups, i.e., presence of LI, in the 0.15 mg/kg MK-801 condition (p<0.05), but not in the vehicle, 0.05, 0.1, and 0.2 mg/kg MK-801 conditions (p>0.05).

Experiment 7: effects of clozapine, D-serine, and ALX 5407 on persistent LI induced by MK-801

Table 6 presents the mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in the ten experimental groups. ANOVA of A periods revealed no differences between the ten groups in their times to complete licks 50–75 before noise onset (all p's >0.05; overall mean A period=9.34 s). Figure 6 shows the mean suppression ratios of PE and NPE groups in five drug conditions: vehicle, MK-801, clozapine + MK-801, D-serine + MK-801, and ALX 5407 + MK-801. ANOVA yielded a significant main effect of preexposure ($F_{1,69}$=5.3, p<0.05) and a significant Preexposure×Drug interaction ($F_{4,69}$=4.3, p<0.01). Post hoc analysis revealed that vehicle-treated mice did not show LI (p>0.05), whereas MK-801-treated

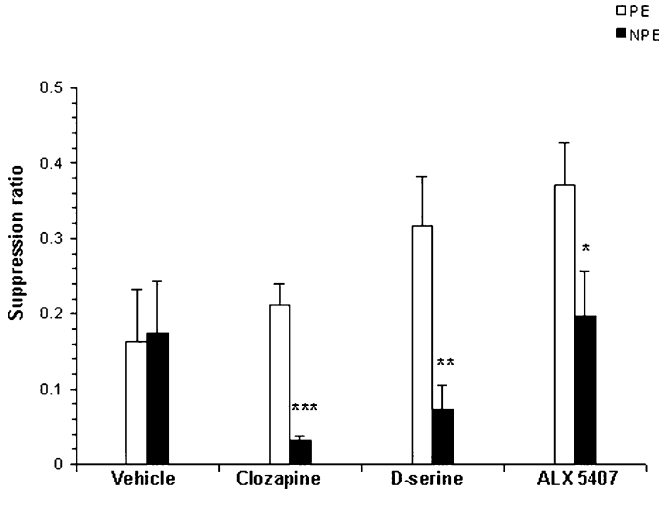

Fig. 4 Mean suppression ratios of the PE and NPE mice in four drug conditions: vehicle, 3 mg/kg clozapine, 600 mg/kg D-serine, and 1 mg/kg ALX 5407 (n=8 per group). Forty noise preexposures and four noise–shock pairings were used. *p<0.05, **p<0.01, ***p<0.001, NPE in comparison with PE score (post hoc LSD test, ANOVA)

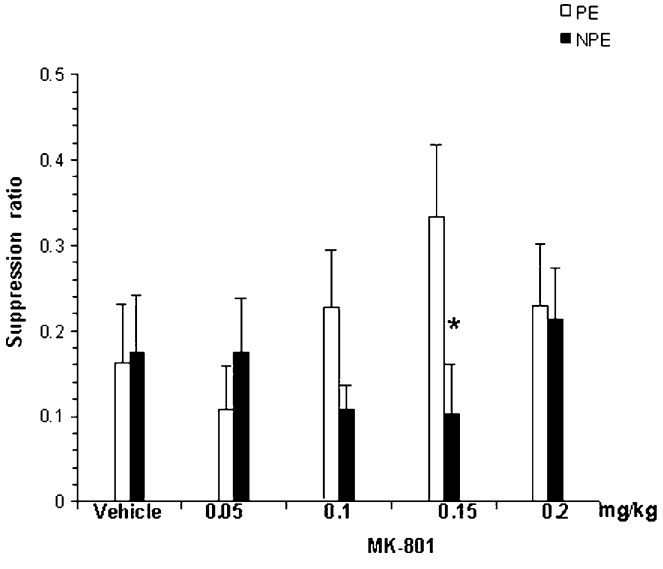

Fig. 5 Mean suppression ratios of the PE and NPE mice in five drug conditions: vehicle, 0.05, 0.1, 0.15, and 0.2 mg/kg MK-801 (n=7–8 per group). Forty noise preexposures and four noise–shock pairings were used. *p<0.05, NPE in comparison with PE score

Table 6 Mean times (in seconds) to complete licks 50–75 (A period) and licks 75–100 (B period) in experiment 7

	A period	B period
Vehicle		
PE	10.0±4.4	90.8±38.6
NPE	7.7±3.9	53.2±19.8
0.15 mg/kg MK-801		
PE	5.6±2.1	8.6±4.2
NPE	9.8±4.4	112.4±32.8
Clozapine + MK-801		
PE	15.9±10.0	59.1±22.2
NPE	13.7±3.4	43.3±14.7
D-Serine + MK-801		
PE	5.6±2.0	12.2±3.8
NPE	13.6±6.3	41.3±9.3
ALX 5407 + MK-801		
PE	5.9±1.6	35.6±11.3
NPE	5.6±1.8	93.5±30.6

mice demonstrated LI ($p<0.001$). MK-801-treated animals that received concomitant injections of clozapine, D-serine, and ALX 5407 showed no LI, similar to vehicle controls ($p>0.05$).

Discussion

The main finding of this study was that agents increasing NMDAR function, via effects at the GMS, potentiated PPI and LI on their own and reversed PPI and LI abnormalities (disrupted PPI and abnormally persistent LI) induced by the NMDA antagonist MK-801 in C57BL/6J mice. Given the great expansion of genetically altered mice and the increasingly central position they occupy in the development of animal models of psychopathology, it is imperative to extend our knowledge of the behaviors of the parental strains used in the creation of these mutants. C57BL/6J mice are commonly used in this capacity, and were chosen for this study because the literature and our pilot investigations indicated that this strain shows moderate levels of PPI, as well as manifests LI. Because the effects of the NMDAR–GMS agents were also produced by the atypical neuroleptic, clozapine, these results support the notion that NMDAR–GMS agents may have an "atypical" antipsychotic profile. Furthermore, the results are consistent with the ability of this class of agents (as well as clozapine) to prevent schizophrenia-like symptoms induced by ketamine in humans, and to ameliorate negative and cognitive symptoms in patients with schizophrenia (Waziri 1988; Goff et al. 1999; Heresco-Levy 2003; Heresco-Levy and Javitt 2004; Tsai et al. 2004). Hence, normal and aberrant PPI and LI may provide model systems that could be useful in evaluating treatments of schizophrenia targeting the glutamate receptor.

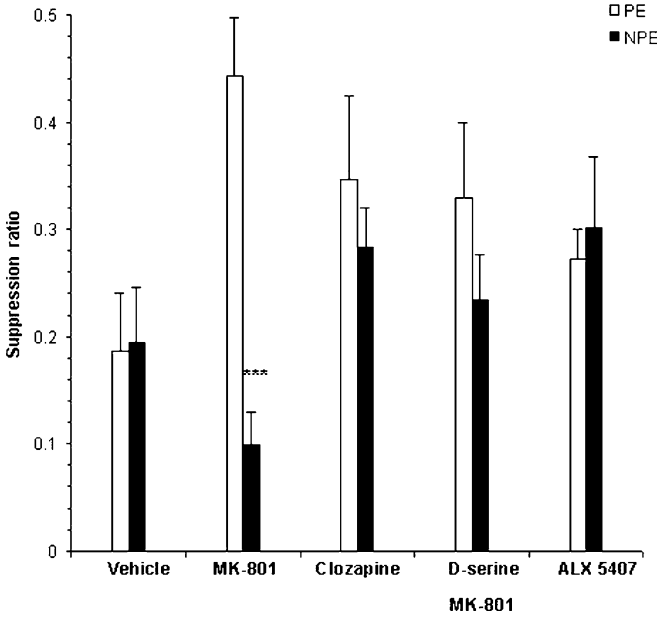

Fig. 6 Mean suppression ratios of the PE and NPE mice in five drug conditions: vehicle, 0.15 mg/kg MK-801, 3 mg/kg clozapine + 0.15 mg/kg MK-801, 600 mg/kg D-serine + 0.15 mg/kg MK-801, and 1 mg/kg ALX 5407 + 0.15 mg/kg MK-801 ($n=8$ per group). Forty noise preexposures and four noise–shock pairings were used. ***$p<0.001$, NPE in comparison with PE score

Prepulse inhibition

Both typical and atypical neuroleptics have been shown to potentiate PPI in mouse strains with low to medium basal levels of PPI, including C57BL/6J mice (Olivier et al. 2001; Ouagazzal et al. 2001). In the present study, clozapine facilitated PPI in C57BL/6J mice at 3 but not at 6 mg/kg. The lack of effect of the higher dose can be attributed to its reducing effect on startle response. This pattern is consistent with previous findings that clozapine enhances PPI and decreases the startle response in C57BL/6J mice (Olivier et al. 2001; Ouagazzal et al. 2001).

D-Serine had no effect on startle at all three doses, and exerted a facilitatory effect on PPI at the 600-mg/kg dose. While the lower dose of D-serine could be a sub-threshold NMDAR activating dose, the high dose may have been ineffective due to hyperactivation of regulatory negative feedback systems or increases in degrading enzymes to compensate for the excessively high levels of D-serine. The effect of D-serine is apparently specific to the glycine binding site because there are no other known neurotransmitter systems affected by this amino acid, including strychnine-sensitive inhibitory glycine receptors (Hashimoto et al. 1993). The effect was specific to the *d* stereoisomer of this amino acid, as L-serine did not enhance PPI.

In contrast to D-serine and clozapine, ALX 5407 did not affect PPI at the lowest dose (1 mg/kg), and moreover, at higher doses reduced PPI. These outcomes are at variance with recent findings showing that ALX 5407 at 1 and 10 mg/kg doses potentiated PPI in DBA/2J mice (Kinney et al. 2003), and could reflect strain differences in the sensitivity to ALX 5407 or in the levels of basal PPI. As for the

higher doses, ALX 5407 apparently acted as an antagonist, producing in the mice increased basal startle reactivity, extreme hyperactivity and strong stereotypy, similar to effects seen after the administration of NMDAR antagonists (Curzon and Decker 1998; Higgins et al. 2003). This notion is consistent with a recent report that high concentrations of the GlyT1 inhibitor CP-802079 lead to a loss of the NMDA augmentation that is seen with lower concentrations of CP-802079 (Martina et al. 2004). Hence, the effect of ALX 5407 at high doses could indeed be associated with a reduction of NMDAR activation.

In line with previous demonstrations of NMDAR antagonist-induced reduction in PPI in rats (Mansbach and Geyer 1989; Bakshi et al. 1994; Varty and Higgins 1995) and mice (Curzon and Decker 1998; Yee et al. 2004), MK-801 disrupted PPI in C57BL/6J mice in a dose-dependent manner, with all the doses exerting maximal effect at the lowest prepulse intensity (69 dB). We also found that clozapine and D-serine exerted maximal PPI facilitation at the same pre-pulse intensity. These findings suggest that C57BL/6J mice may be most sensitive to drug influences at the lowest prepulse intensity, as found by others (Ouagazzal et al. 2001). As would be expected from previous studies (Bakshi et al. 1994; Varty and Higgins 1995) MK-801 induced PPI disruption was reversed by clozapine. The sensitivity of the NMDAR antagonist-induced PPI disruption, or the so-called "PCP–PPI model", to clozapine and other atypical neuroleptics, is considered a major strength of this model insofar as it is believed that it may reveal information that is relevant to treatment-resistant patients. Surprisingly, despite growing evidence that drugs enhancing glycine binding site function can be beneficial in such schizophrenia patients (Waziri 1988; Goff et al. 1999; Heresco-Levy 2003; Heresco-Levy and Javitt 2004; Tsai et al. 2004), to the best of our knowledge, there have been no studies testing the effects of pro-glycine treatments on NMDAR antagonist-induced PPI disruption, although glycine and the GlyT1 inhibitor ORG 24598 were recently shown to reverse a disruption in PPI caused by a neonatal ventral hippocampal lesion (Le Pen et al. 2003). Our results provide the first demonstration that NMDAR antagonist-induced PPI disruptions can be reversed by agents potentiating NMDAR transmission via effects at the GMS. Of the two such agents used, the highly selective GlyT1 inhibitor, ALX 5407 reversed the MK-801 induced disruption of PPI whereas D-serine showed only a trend to normalize the impaired PPI. This is consistent with the expectation and findings that increasing synaptic glycine levels by inhibition of glycine reuptake systems should produce a more significant potentiation of NMDAR-mediated neurotransmission than is possible to obtain with exogenous glycine/D-serine/administration (Javitt 2002). However, it should be noted that the same dose of ALX 5407 failed to potentiate PPI when given on its own. Thus, it is possible that the effect of increased synaptic levels of glycine differ depending on channel activation state of the NMDA receptors.

Latent inhibition

In this study, we demonstrated that intact C57BL/6J mice that received 40 noise preexposures and two noise–shock conditioning trials, showed LI, but when the same number of preexposures was followed by four conditioning trials, LI was disrupted. This mimics the reported disruption of LI in rats by extended conditioning in the same Conditioned Emotional Response procedure (Weiner et al. 1996, 1997, 2003; Shadach et al. 2000). Neuroleptics on their own have been repeatedly shown to enhance LI in rats under parametric conditions that reduce or disrupt LI in controls (Weiner et al. 1996, 1997, 2003; Moser et al. 2000; Shadach et al. 2000). This effect is specific and selective for drugs with known antipsychotic activity and is not produced by a wide range of nonantipsychotic drugs (Dunn et al. 1993). Consistent with this body of literature, the present study demonstrated that clozapine potentiates LI in mice, and shows for the first time that the same effect is produced by D-serine and ALX 5407. This behavioral similarity suggests that agents enhancing GMS function may share common features with neuroleptics.

While drug-induced disrupted LI in the rat is considered to model positive symptoms of schizophrenia (Weiner 1990, 2003; Gray et al. 1991; Moser et al. 2000), LI can exhibit an opposite pole of abnormality, whereby it persists under conditions that normally disrupt LI, and it was suggested that persistence of LI may reflect impaired set shifting, that is associated with cognitive inflexibility and negative symptoms (Weiner 2003). Recently, excessive LI has been demonstrated in schizophrenia patients, and, most importantly, has been shown to positively correlate with the level of negative symptoms (Rascle et al. 2001; Cohen et al. 2004). Gaisler-Salomon and Weiner (2003) provided the first results showing that systemic administration of MK-801 at low dose induces abnormally persistent LI, which can be reversed by the atypical antipsychotic drug clozapine, but not by the typical antipsychotic haloperidol. In line with this finding, we showed that under the LI disrupting parameters of extended conditioning, C57BL/6J mice treated with a low (0.15 mg/kg) dose of MK-801 have persistent LI. The two lower doses were ineffective, whereas the highest dose impaired conditioning. A similar narrow dose–response curve was observed by Gaisler-Salomon and Weiner (2003), although in their study, persistent LI was obtained with 0.05 mg/kg. Due to the well-documented propensity of NMDA antagonists to impair or abolish associative learning (Bardgett et al. 2003), low doses of MK-801 that do not impair conditioning in the non-preexposed rats are imperative for producing persistent LI. This is because the emergence of the LI effect, namely, poorer conditioning of the preexposed compared to non-preexposed rats, is only possible if the drug does not impair conditioning in the non-preexposed group. Moreover, it can be seen in Figs. 5 and 6 that MK-801 affected the non-preexposed and the preexposed groups in an opposite manner, decreasing conditioning in the PE group but increasing conditioning in the NPE group. This distinct effect of a low MK-801 dose on conditioning to a new stimulus and to a

stimulus with which the animal had previous experience, is similar to the recent findings of van der Meulen et al. (2003) which show that MK-801 disrupted discrimination reversal learning without impairing discrimination learning, and that the selective effect of MK-801 on reversal learning was obtained with low doses (0.025 and 0.05) but not with a higher (0.1 mg/kg) dose.

MK-801-induced persistent LI in mice was reversed by clozapine, as well as by D-serine and ALX 5407, such that MK-801-treated animals given these drugs showed no LI, as did vehicle-treated mice. Thus, data obtained from LI experiments demonstrate that direct and indirect modulators of the GMS, as well as clozapine, are able to regulate the two poles of abnormality in LI, which may be associated with positive and negative symptoms of schizophrenia.

PPI and LI: commonalities and differences

Glycinergic agents acted more consistently in LI as both potentiated LI and reversed MK-801-induced persistent LI. However, in the case of PPI, potentiation was produced by D-serine but not ALX 5407, and vice versa for MK-801 induced disrupted PPI. The lower dose of MK-801 used in LI compared to PPI may explain why in the case of LI D-serine was effective in reversing MK-801 effects. As for potentiation, the robustness of this effect clearly depends on the basal level of the assessed behavior in the controls. While control C57BL/6J mice showed a medium basal level of PPI, the controls in the LI experiments showed no LI at all because of parametric manipulation to ensure its loss. Interestingly, we have identified the mouse strain with *spontaneously* low levels of LI, and found that LI is potentiated in this strain by both typical and atypical antipsychotics (unpublished results).

Although clozapine affects multiple receptors (Meltzer and Nash 1991), reversal of NMDAR antagonist induced PPI disruption by clozapine has been attributed to its antagonistic action at the 5-HT_2 receptor, because the same effect is produced by selective $5HT_{2A}$ antagonists (Bakshi et al. 1994). The same has been argued for clozapine's ability to reverse MK-801-induced persistent LI (Gaisler-Salomon and Weiner 2003). If this is the case, then the interaction between clozapine and NMDAR antagonists with regard to their effects on PPI and LI is not due to a competition for a common receptor because NMDAR antagonists do not have an appreciable affinity for $5HT_{2A}$ receptors. In contrast, D-serine and ALX 5407 certainly do act to produce their behavioral effects on LI and PPI via activation of the NMDAR. Indeed, both are very selective for the glycine binding site (Hashimoto et al. 1993; Atkinson et al. 2001).

It remains to be elucidated how GMS stimulation interferes with the action of MK-801. GMS stimulation increases the frequency and duration of NMDAR channel opening (Bonhaus et al. 1989; Sheinin et al. 2001; Millan 2002), and in this manner may promote the dissociation of MK-801 from the receptor pore (von Euler and Liu 1993; Millan 2002). Alternatively, GMS stimulation may recruit and activate a larger proportion of NMDA receptors (Danysz and Parsons 1998), thus "diluting" the effects of NMDAR blockade by MK-801. It should be noted that GMS stimulation could also be expected to *enhance* MK-801 effects due to an increased ability of MK-801 to reach the open channel site. Indeed, increased MK-801/PCP binding and MK-801 induced locomotor activity following treatment with GlyT1 inhibitors has been reported (Tedford et al. 2002; Waterhouse 2003). The fact that MK-801 produced here behavioral effects when given on its own indicates that a sufficient proportion of channels were stimulated (in the open state) to allow MK-801 to block the channels. Under such conditions, treatments that induce GMS activation may serve to release the bound antagonist. Whether the opening of the NMDA channel caused by GMS stimulation promotes the "invasion" of MK-801 or its "ejection" is likely to depend on many factors, including the doses of agonists and antagonists employed and possibly the behavior assessed. Moreover, since MK-801 not only blocks the NMDAR, but also induces glutamate release resulting in activation of other glutamate receptors (AMPA and kainate) (Bredt and Nicoll 2003), its behavioral effects can be ultimately due to a deficit or an excess of glutamatergic transmission. It was recently shown that ketamine-induced disrupted PPI was reversed by lamotrigine (Brody et al. 2003), which decreases glutamate release, indicating that the ketamine-induced effect is mediated at least in part by excessive glutamatergic transmission. It remains to be investigated whether the effects of MK-801 on PPI and LI are in part also mediated by excessive glutamatergic transmission, as has been found for ketamine-induced psychosis in humans (Krystal et al. 2003).

Finally, in recent years, attention has been directed to the ability of clozapine to act as a partial agonist at the GMS (Arvanov et al. 1997; Schwieler et al. 2004), and this mechanism has been suggested to underpin its unique actions in the clinic and in animal models (Goff and Coyle 2001; Coyle et al. 2003). The present finding that clozapine effects are mimicked by pro-glycine treatments, supports this possibility. However, this remains to be investigated. In rats, we found that although clozapine and glycinergic treatments reversed MK-801 induced LI persistence, their effects were distinct, with the former acting at the pre-exposure stage and the latter acting at the conditioning stage (unpublished results).

Although PPI and LI appear to be modulated in a similar manner, the relationship between the drug-induced potentiation and reversal of MK-801 induced aberration is different in PPI and LI. Potentiation of PPI and reversal of MK-801 induced PPI disruption reflect a common process, namely, in both cases, the drugs *strengthen* PPI. The ability of clozapine and glycine binding site agonists to reverse the pharmacologic disruption of PPI may be at least partly accounted for by their capacity to potentiate PPI, and both phenomena are likely to be subserved by common mechanisms. This is not the case with LI. Here the drugs *potentiate* LI when given alone but *disrupt* LI when given with MK-801. Therefore, the effects on LI exerted by these

drugs on their own and in conjunction with MK-801, likely reflect interactions with distinct neural pathways or systems within the complex forebrain circuitry that regulates LI (Weiner 2003). In other words, LI potentiation produced by clozapine and NMDA agonists may depend on actions within brain sites that differ from the sites at which these drugs disrupt MK-801-induced LI. If persistent LI is confirmed to be a behavioral aberration that models well schiophrenia-like pathology, then persistent LI in MK-801-treated mice might be a sensitive approach to uncover novel mechanisms of antipsychotic action.

Conclusion

The suggestion that potentiation of NMDAR function may be useful for the treatment of schizophrenia is derived from the notion that NMDAR hypofunction may be critically involved in the etiology or pathophysiology associated with this disorder. More specifically, with regard to the role of glycine, recent genetic evidence suggests that a polymorphism in a primate-specific gene (G72) may be linked to schizophrenia. The protein coded by G72 positively modulates D-amino acid oxidase, which in turn metabolizes D-serine (Chumakov et al. 2002). These findings raise the possibility of a primary deficiency of the NMDAR-dependent glycine system in schizophrenia, supplementing the clinical findings that agonists at the glycine-binding site are beneficial in patients with schizophrenia (Waziri 1988; Goff et al. 1999; Tsai et al. 2004; Heresco-Levy and Javitt 2004). The present results show the ability of PPI and LI models to detect the effects of such agents, although the sensitivity of these models may vary due to the involvement of different neuronal pathways. Since only single doses were used in most of the experiments and in only one mouse strain, the present results should be considered preliminary. However, this study is novel in demonstrating that D-serine and ALX 5407 exert in mouse models of PPI and LI behavioral effects considered relevant to the treatment of schizophrenia, supporting the claim that the GMS may be a target for novel antipsychotics.

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